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Effects of experimental acidification on nematode populations in soil cultures

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With 3 figures

Synopsis: *Original scientific paper*

The effect of artificial acidification on soil nematodes were determined in a laboratory experiment. Soil samples were sprayed with diluted sulfuric, nitric or oxalic acid of *p*H 2.0, 1.5 and 1.0 and incubated for 6 weeks.

The number of bacteria decreased after acid treatment. The response to all tested acids was similar.

Acidification caused negative effects on nematode species number and population density. Additionally, the dominance structure changed. A shift in favour to root and fungal feeders was observed along with increasing acid concentrations. The effects of oxalic acid were of short duration.

Among the root and fungal feeders the species of the genus *Aphelenchoides* dominated after acidification. Comparing two bacterial feeders, the eudominant species *Wilsonema schuurmansstekhoveni* (DE CONICK, 1931) and the rare species *Acrobeloides buetschlii* (DE MAN, 1884), the latter gained in importance after the acid treatment.

Key words: soil nematodes, acidification effects, bacterial feeders, root and fungal feeders.

1. Prefatory note

Distribution and abundance of soil nematodes are influenced by soil moisture, temperature and acidity. The *p*H-value greatly influences soil microflora, which is a substantial diet for many soil animals.

Effects of experimental acidification on the nematode abundance have already been reported by HEUNGENS (1981), HUHTA *et al.* (1986) and HÅGVAR (1987). These studies indicate that nematodes could be sensitive not only to increased, but also to decreased soil acidity.

The purpose of the here presented investigations was to determine the effects of various acids and soil *p*H-values on nematode species range, abundance, dominance and trophic structure. One object was to determine whether particular groups or species benefitted from or were suppressed by acidification. Additionally, the response of bacteria, which are a food source for Nematoda, to acid treatments was investigated.

2. Materials and methods

Soil samples were taken in a ca 70 years old spruce stand at Ulm (South-West Germany, for details see FUNKE, 1986). The study was conducted using the humus and the litter layer ($O_{l+f}/O_h + A_h$). The ground vegetation was scarce and only isolated spots were covered by mosses.

The soil samples were mixed prior to culturing in order to compensate for the natural variation in chemical properties of the undisturbed soil. Glass Petri dishes (150 pieces, ∅15 cm) were each filled with 40 g (fresh mass) of soil substratum. The control samples (15 dishes) were sprayed with 20 ml of distilled water. Diluted solutions (20 ml) of sulfuric, nitric or oxalic acid (*p*H 2.0, 1.5 and 1.0) were

applied for acidification. Each treatment was done with three replicates. The Petri dishes were incubated in a climatic chamber. The daily cycles were kept with 10 h of light at 18 °C and 14 h of darkness at 15 °C.

Sampling took place 1, 2, 3, 4 and 6 weeks after application. Each time one sample of 10 g soil was taken from certain Petri dishes for nematode extraction. The remaining mixed soil substratum was used for measurements of *p*H-values (in KCl 1 molar). The alterations during the experimental period are listed in table 1.

Nematodes were extracted in modified wet funnels (BAERMANN, 1917). A temperature gradient was induced starting after 24 hours at 20 °C and ending after 6 hours at 45 °C. Afterwards the substratum was drained. Following the extraction the nematodes were preserved in 4% formaldehyde solution and total numbers were counted. Of the nematodes counted 10% (a total of 32115 animals) were identified at species level.

In a separate experiment under similar conditions the responses of soil bacteria were tested. The number of bacteria was obtained by counting on a fluorescence microscope using acridine orange as a stain (see DUNGER & FIEDLER, 1989).

Student's t-test was used to test significant influences of treatments on the number densities of bacteria or Nematoda.

Table 1. Changes in *p*H-values of the soil cultures 1, 2, 3, 4 and 6 weeks after acid application.

Week	Control	H_2SO_4			HNO_3			Oxalic Acid		
		2.0	1.5	1.0	2.0	1.5	1.0	2.0	1.5	1.0
1	3.0	2.9	2.8	2.3	3.0	2.9	2.5	3.1	3.1	2.2
2	3.0	3.0	2.8	2.3	3.0	2.9	2.6	3.2	3.1	2.8
3	3.0	3.0	2.9	2.4	3.0	2.9	2.6	3.1	3.1	3.0
4	3.0	3.0	2.8	2.3	3.0	2.9	2.6	3.1	3.0	3.3
6	3.0	3.0	2.8	2.3	3.0	3.0	2.6	3.1	3.0	3.3

Note: 2.0, 1.5, 1.0-*p*H of the applied acid solutions.

3. Results

3.1. Effects of acidification on bacteria

At the beginning of the experiment acidification caused negative effects on the number of bacteria in almost all cultures (table 2). The number of bacterial cells declined with increasing acid concentrations. During the experimental period their number increased in the controls and in the samples treated with acid solutions of *p*H 2.0. Over the course of the experiment higher concentrated acid solutions reduced the bacterial numbers significantly.

Table 2. Number of bacteria per g dry soil ($\times 10^9$) during the culture experiment.

Week	Control	H_2SO_4			HNO_3			Oxalic Acid		
		2.0	1.5	1.0	2.0	1.5	1.0	2.0	1.5	1.0
1	3.49	2.91	2.53*	1.02**	2.58*	2.07*	1.28**	2.25*	2.11*	1.29**
2	3.47	2.43*	2.06*	1.03**	2.42*	1.91*	0.93**	2.36*	2.39*	1.20**
3	3.77	3.39	2.50**	1.60***	2.85**	1.85**	1.51***	2.67**	2.04***	1.76**
4	4.59	4.42	2.27**	1.16**	3.96	1.70**	0.99**	3.66	2.48*	1.41**
6	6.39	7.10	2.88	1.60*	5.57	3.31	0.90*	5.02	3.29	1.54*

Note: 2.0, 1.5, 1.0-*p*H of the applied acid solutions. *, **, ***, with $P \leqq 0.05$, $P \leqq 0.01$, or $P \leqq 0.001$ significantly different from the control.

3.2. Nematode species range and population density

In former studies 49 species were identified at the experimental site (FUNKE, 1991). In the cultures 30 different species were found (table 3), 21 of them were bacterial and 7 root and fungal feeders. Only one species belonged either to the trophic group of omnivores or predators.

From the observed nematode species 20 showed a negative reaction to acid applications. Comparing cultures contaminated with diluted acids (*p*H 2.0) to controls no changes in the species range were found. Along with the increasing acid concentrations (*p*H 1.5, 1.0) species numbers declined. This decline affected, besides the predator *Prionchulus paucidentátus*, almost exclusively bacterial feeders (table 3).

At the beginning of the experiment significant reductions of the population densities were only recorded at acid solutions of *p*H 1.0 (fig. 1). During the course of 4 – 6 weeks

Table 3. Nematode species of which individuals outlived the acidification of their living substrate during a period of 1 – 6 weeks after application.

	Control	H_2SO_4			HNO_3			Oxalic Acid		
		2.0	1.5	1.0	2.0	1.5	1.0	2.0	1.5	1.0
Bacterial feeders										
Plectus geophilus DE MAN, 1880	+	+	+	.	+	+	+	+	+	+
Metateratocephalus crassidens (DE MAN, 1880)	+	+	+	.	+	+	+	+	+	
Panagrobelus emarginatus (DE MAN, 1880)	+	+	+	.	+	+	.	+	+	
Alaimus primitivus DE MAN, 1880	+	+	+	.	+	+	.	+	+	
Prismatolaimus intermedius (BÜTSCHLI, 1873)	+	+	+	.	+	.	.	+	+	
Plectus communis BÜTSCHLI, 1873	+	+	+	.	+	.	.	+		
Eumonhystera vulgaris (DE MAN, 1880)	+	+	.	+	+	.	.	+	+	
Bunonema reticulatum RICHTERS, 1905	+	+	.	.	+	.	+	+	+	
Plectus acuminatus BASTIAN, 1865	+	+	.	.	+	.	.	+	+	
Teratocephalus terrestris (BÜTSCHLI, 1873)	+	+	.	.	+	+				
Rhabditis silvatica VOLZ, 1951	+	+								
Bunonema ditlevseni MICOLETZKY, 1925	.	.	+	.	.	.	.	+		
Ceratoplectus armatus (BÜTSCHLI, 1873)	.	.	.	.	+	.	.	+		
Bunonema richtersi JÄGERSKJÖLD, 1905	.	+								
Rhabditis terricola DUJARDIN, 1845	.	.	.	.	+					
Prismatolaimus dolichurus DE MAN, 1880	.	.	.	.	.	.	.	.	+	
Root and fungal feeders										
Aphelenchoides lagenoferrus BARANOVSKAYA, 1963	+	+	+	+	+	.	+	+	+	+
Malenchus bryophilus (STEINER, 1914)	.	+	.	.	.	.	.	+		
Omnivores										
Eudorylaimus discolaimioideus (ANDRÁSSY, 1958)	+	+	+	.	+	+	.	+	+	+
Predators										
Prionchulus paucidentatus ZELL, 1985	+	.	.	.	+	.	.	.	+	

Note: Different *p*H (2.0, 1.5, 1.0) of the applied acid solutions. + species observed in the cultures.

Species of which individuals outlived all treatments.

Bacterial feeders:

Acrobeloides buetschlii (DE MAN, 1884); *Eucephalobus oxyuroides* (DE MAN, 1876); *Teratocephalus paratenuis* EROSHENKO, 1973; *Teratocephalus stratumus* EROSHENKO, 1973; *Wilsonema schuurmans-stekhoveni* (DE CONICK, 1931).

Root and fungal feeders:

Aphelenchoides macronucleatus BARANOVSKAYA, 1963; *Aphelenchoides parasubtenuis* SHAVROV, 1967; *Ditylenchus intermedius* (DE MAN, 1880); *Filenchus istvani* ZELL, 1988; *Filenchus longicaudatulus* ZELL, 1988.

the numbers of individuals increased in all cultures. In this period the proportion of juveniles in the total number of nematodes rose from 53–70% to 66–86%. In some cultures with low concentrated acids nematode numbers were higher than in the controls. This effect occurred particularly following the contamination with oxalic acid. In those cultures the population density was compared to all others, significantly higher four weeks after application of *p*H 2.0 solutions. Until the end of the 4th week a dependency of the population density on the acid concentration was observed in all treated cultures. After 6 weeks of cultivation the population density remained significantly reduced in samples treated with sulfuric acid (*p*H 2.0 and 1.5), nitric acid (*p*H 1.5 and 1.0) and oxalic acid (*p*H 1.5).

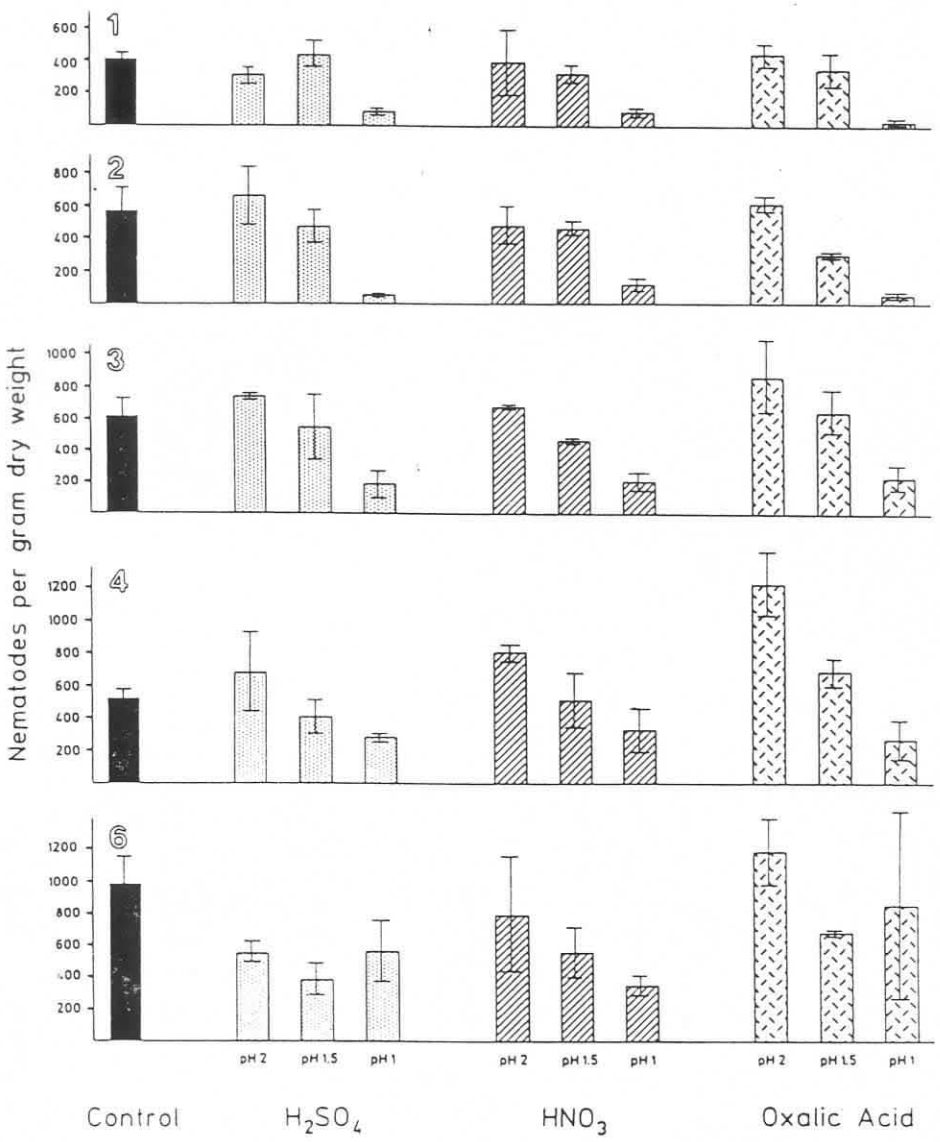

Fig. 1. Influence of acidification on nematode population density (nematodes per gram dry weight of soil substratum ± s.d.). Samples taken after 1, 2, 3, 4 and 6 weeks.

3.3. Trophic structures of the nematodes (according BANAGE, 1963; YEATES, 1971; & SMALL, 1987)

The proportion of bacterial feeding nematodes was always >50% in the controls and under the influence of diluted acids (fig. 2; *p*H of soil cultures 2.9–3.2, table 1). The proportion of bacterial feeders declined with increasing acid concentrations. During the course of the experiment this effect was even more evident under the influence of H_2SO_4 and HNO_3. The reduction of bacterial feeders was significant beginning the 2nd week after applying solutions of *p*H 1.0 and beginning the 3rd week after applying solutions of *p*H 1.5 until the end of the experiment. However, during the same period the bacterial feeders recovered following the oxalic acid applications (*p*H 1.0). These results are likely coupled with the increasing soil *p*H-value from 2.2 to 3.3 (table 1).

Entirely opposite to this was the reaction of the root and fungal feeders (fig. 3). Their numbers increased significantly along with the acidification and the duration of the experiment under the influence of H_2SO_4 and HNO_3 (*p*H 1.5 and 1.0). Increasing numbers of individuals belonging to the genus *Aphelenchoides* appeared. At the end of the experiment

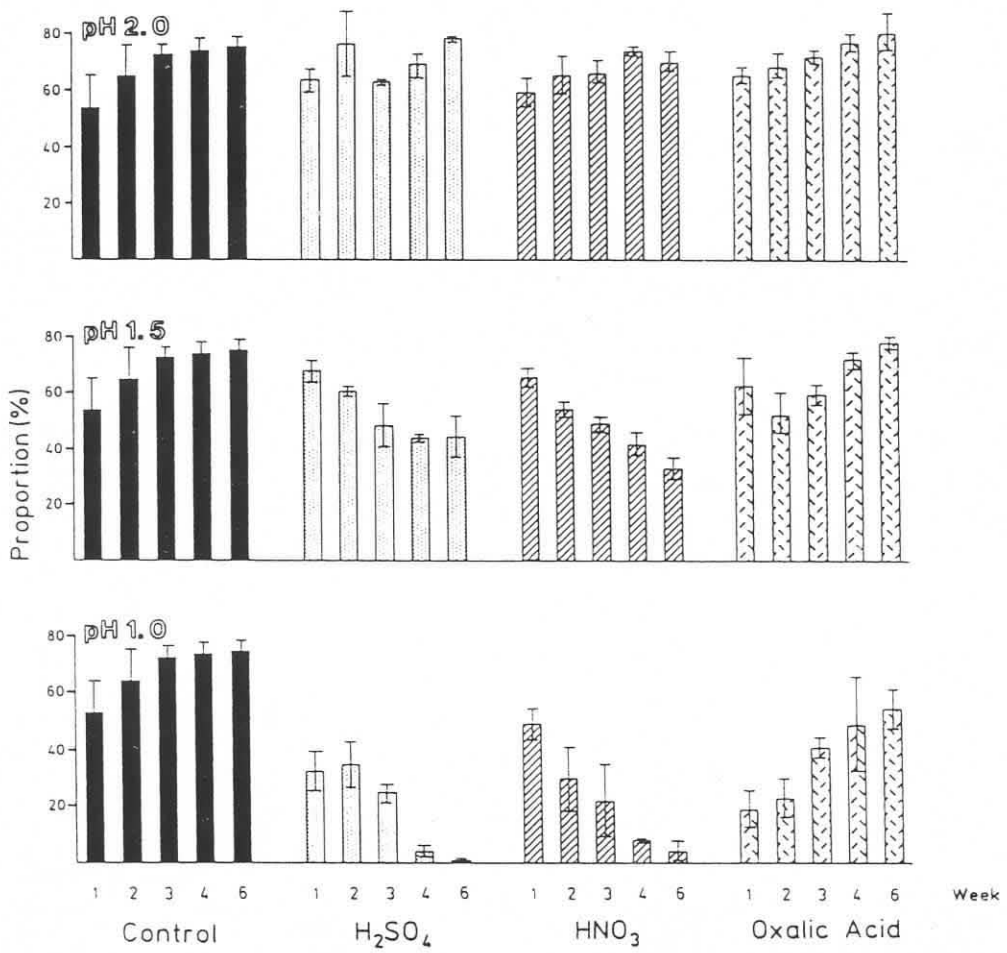

Fig. 2. The proportion (± s.e.) of the bacterial feeding nematodes after treatment with acids of *p*H 2.0, 1.5 and 1.0 during the experimental period of 6 weeks.

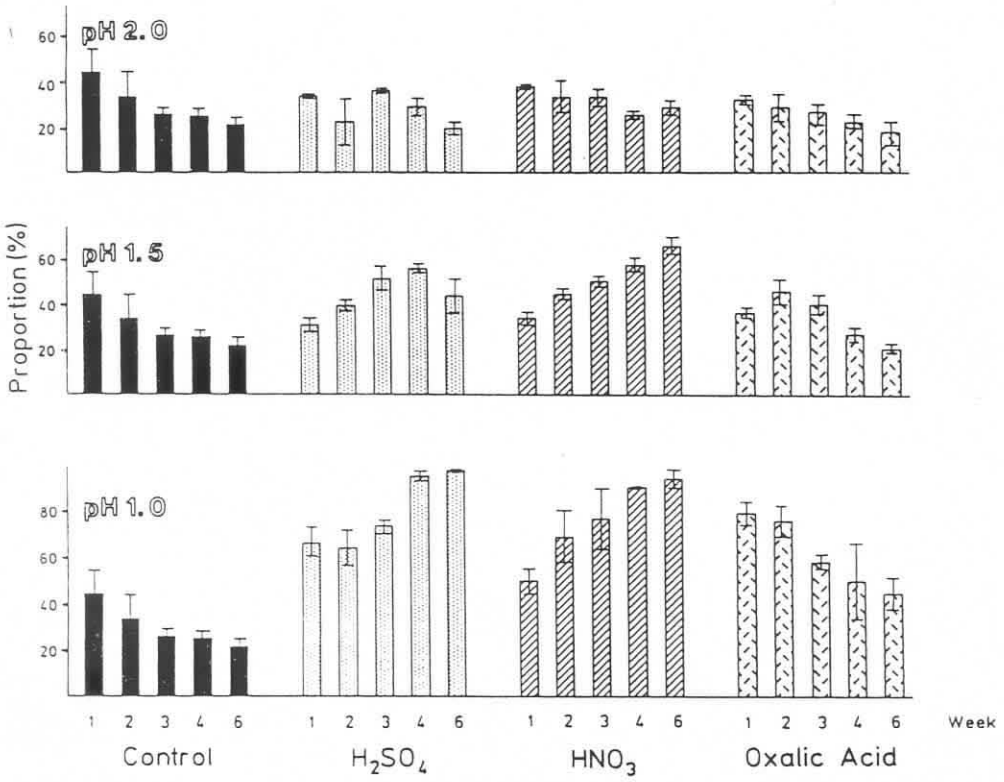

Fig. 3. The proportion (± s.e.) of root and fungal feeding nematodes after treatment with acids of *p*H 2.0, 1.5 and 1.0 during the experimental period of 6 weeks.

Table 4. The percentage of *Wilsonema schuurmansstekhoveni* (A) and *Acrobeloides buetschlii* (B) during the experimental period.

	Week	Control	H_2SO_4			HNO_3			Oxalic Acid		
			2.0	1.5	1.0	2.0	1.5	1.0	2.0	1.5	1.0
A	1	17.8	22.2	22.5	10.7*	23.6	21.2	30.7	23.3	27.0	7.9
	2	24.0	25.7	24.2	9.0	30.1	21.4	16.5	30.0	24.4	4.7
	3	44.9	34.5*	13.2**	6.4**	36.4	15.5**	15.5**	43.6	32.1*	6.0**
	4	50.8	44.8	13.2**	1.9***	50.5	12.2**	6.3***	53.7	45.5	9.0**
	6	57.4	57.3	8.9***	0.5***	48.7	11.1**	3.5***	65.8	60.5	5.8**
B	1	1.2	1.4	2.4	1.7	3.9	2.9	1.7	1.1	2.0	3.3
	2	4.6	4.5	6.0	4.3	8.0	5.5	1.8	8.1	4.6	11.0
	3	4.9	11.0	15.5*	7.2	7.3	11.3*	1.2	8.1	8.3	23.5**
	4	6.2	8.8	13.7*	1.1*	8.6	15.1	0.7*	7.9	11.5	29.2*
	6	2.3	3.9	10.5**	0.4	8.6*	13.8**	—	3.9	7.0	46.1**

Note: 2.0, 1.5, 1.0-*p*H of the applied acid solutions. *, **, ***, with $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$ significantly different from the control.

these species totaled more than 90% of all individuals. The numbers of root and fungal feeders decreased along with the rising *p*H-value in the cultures treated with oxalic acid (*p*H 1.0).

The omnivores and predators were not taken into consideration in these studies. Their percentage of the total nematode populations stayed under 3.5%. Acid applications led to a total disappearance of the predator *P. paucidentatus* (table 3).

At the beginning of the experiment *Wilsonema schuurmansstekhoveni* was the dominant bacterial feeder in almost all cultures (table 4). Its percentage increased in the controls and under the influence of low concentrated acids (*p*H 2.0 of all acids and additionally of oxalic acid *p*H 1.5) from 17.8–27% to 48.7–65.8%. Concentrated acids of H_2SO_4 and HNO_3 (*p*H 1.5, 1.0) and oxalic acid (*p*H 1.0) reduced the percentage of *W. schuurmansstekhoveni*. These effects were significant after three weeks of incubation.

At first no changes in the dominance position of another bacterial feeder (*Acrobeloides buetschlii*) were noticeable (table 4). Over time and under the influence of sulfuric or nitric acid (*p*H 1.5) and mainly of oxalic acid (*p*H 1.0) its importance increased significantly.

4. Discussion

Experimental acidification may influence soil chemistry in different ways. The soil water contains a wide variety of ions and is highly buffered. Addition of acids may cause an increased ion exchange in the soil, which in turn results in a higher osmotic potential of the soil water. Water living animals may have difficulties with the salt uptake (BÅÅTH *et al.*, 1980). Our results indicate that the effect of sulfuric and nitric acid on soil *p*H is similar. These inorganic acids are relatively stable in water solution. In comparison it is possible for oxalic acid to build complexes with calcium and potassium ions or to become oxidized. In higher concentrations it turns into a cell toxin.

Acid solutions of *p*H 2.0 and 1.5 had little influence on the soil *p*H. However, a negative effect on species number and population density of nematodes became partly noticeable following the treatment with acid solutions of *p*H 2.0 and even more evident with *p*H 1.5 and 1.0. The effect of oxalic acid on population densities was lower and did not last as long as for the inorganic acids. Similar effects were observed by HEUNGENS (1981) during a treatment of pine litter with nitric and citric acid.

The soil microflora responds to the reduction of the soil *p*H with a change in favour of fungi. The soil *p*H is evidently correlated with the density of fungal hyphae and the number of bacteria (see BÅÅTH *et al.*, 1980; FEEST & CAMPBELL, 1986). The number of bacteria decreased in the cultures after acid treatment and a decline of nematode species was observed mainly within the bacterial feeders. Additionally, the shift from bacterial feeding to root and fungal feeding nematodes reflected the changes in the dominance structure of the microflora. These observations correspond with those of BURNS (1971) and HEUNGENS (1981).

The raising *p*H-value in the samples treated with concentrated oxalic acid caused an increase of the proportion of the bacterial feeders and simultaneously a decrease of the root and fungal feeders. Similar effects on the trophic structure of the nematodes were caused by a *p*H-value increase following fertilization (HUHTA *et al.*, 1983). An increase of the number of bacteria after treatment with oxalic acid was not observed in the second experiment. Some other influences, like the soil moisture, could have been more important.

Changes of soil *p*H-value possibly alter the nematode population directly, e.g. its reproduction ability (LEHMAN *et al.*, 1971) or it may also cause indirect effects. Besides other influences, these indirect alterations are particularly caused by a change of the amount and the availability of food for certain species (see NICHOLAS, 1962). These may effect the dominance structure within trophic groups. Additionally, a change in the *p*H-value may lead to a reduction of predators and consequently this can lead to diverse effects on potential

prey species. All these effects may cause the promotion of well adapted species and the suppression of others at the same time (see HÅGVAR & ABRAHAMSEN, 1980; YEATES, 1981). The strong increase of the genus *Aphelenchoides* within the root and fungal feeders as well as the increase of the bacterial feeder *A. buetschlii* after acid application was likewise observed in a field experiment at the same spruce forest (RATAJCZAK *et al.*, 1989). HYVÖNEN & PERSSON (1990) also reported that the genus *Aphelenchoides* might be more tolerant to high acid concentrations than most other nematodes.

Comparing two bacterial feeders, the eudominant species *W. schuurmansstekhoveni* and the initially rare species *A. buetschlii*, the latter gained in importance following the treatment with sulfuric and nitric acid solutions of *p*H 1.5 (table 4). The change of the soil *p*H within four weeks from 2.2 to 3.3 in cultures treated with oxalic acid (*p*H 1.0, see table 1) kept *W. schuurmansstekhoveni* at relatively low levels, while *A. buetschlii* reproduced well. Evidently it is possible for *A. buetschlii* to adapt faster and better than *W. schuurmansstekhoveni* to rapid *p*H changes and therefore to altered food availability. Similar effects may be expected for many other species. It is generally known that the dominance structure of a coenosis depends on competition. Here the decline of one species is often compensated by the increase of another species (SOHLENIUS, 1985).

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6. Zusammenfassung (Auswirkungen experimenteller Säuregaben auf Nematodenpopulationen in Bodenkulturen)

In einem Laborexperiment wurden die Auswirkungen von künstlicher Säurebehandlung auf Bodennematoden untersucht. Bodenproben wurden mit verdünnter Schwefel-, Salpeter- oder Oxalsäure von *p*H 2,0, 1,5 und 1,0 besprüht und 6 Wochen inkubiert.

Die Anzahl der Bodenbakterien ging nach Säurekontamination zurück. Die Reaktionen waren bei allen Behandlungen ähnlich.

Die Säurebehandlung hatte negative Effekte auf Artenzahl und Wohndichte der Nematoden. Daneben kam es auch zu Veränderungen in der Dominanzstruktur. Der Anteil der Wurzel- und Pilzfresser stieg mit der Säurekonzentration deutlich an. Dabei waren die Auswirkungen von Oxalsäure nur von kurzer Dauer.

Innerhalb der Wurzel- und Pilzfresser dominierte nach Säuregaben die Gattung *Aphelenchoides*. Beim Vergleich zweier Bakterienfresser, der eudominanten *Wilsonema schuurmansstekhoveni* (DE CONICK, 1931) und der selteneren Art *Acrobeloides buetschlii* (DE MAN, 1884), gewann letztere nach Säurebehandlung an Bedeutung.

Schlüsselwörter: Bodennematoden, Säuregaben, Bakterienfresser, Wurzel- und Pilzfresser.

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